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L2: Entry 1 of 270 File: USPT May 27, 2003

DOCUMENT-IDENTIFIER: US 6569418 B1
TITLE: Immuno-modulating effects of chemokines in DNA vaccination

Detailed Description Text (119):
The humoral response against gp120, when injected alone was substantially higher than the vaccination with gp160 alone, when following the same infection regimen, which indicates that the use of a soluble antigen modulates the immune response that leads to an increased Ab production. This result demonstrates the significance of the form of the antigen used. Furthermore, a significant increase in the antigen-specific Ab titer was detected after the second injection for both the soluble antigen gp120 as well as the membrane-bound antigen gp160, but this changed after the third injection. Both titers declined. This finding suggests the importance of the regimen and informs future vaccination strategies.

hydrophobic

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L2: Entry 37 of 270 File: USPT Mar 19, 2002

DOCUMENT-IDENTIFIER: US 6358718 B1
TITLE: Method for stabilization and renaturation of proteins using nucleolar protein B23

Detailed Description Text (36):
At low ionic strength the Rev protein undergoes a slow polymerization in a temperature-independent manner with formation of insoluble fibrous material (Wingfield et al., 1991). To test whether protein B23 also inhibited the temperature-independent polymerization of Rev, aliquots of Rev in high ionic strength buffer (approximately 400 mM) were dialyzed in micro dialyzer cassettes against low ionic strength buffer (20 mM) at 4.degree. C. The samples included various concentrations of protein B23.1. The turbidity was measured as above after overnight dialysis. The samples containing Rev alone showed the greatest level of turbidity, whereas addition of increasing concentrations of protein B23 resulted in decreasing turbidity (FIG. 1C). As with the temperature-dependent aggregation described above the solutions were essentially clear when the B23:Rev ratio was greater than one. The Rev protein also has a tendency to polymerize and form filaments over a period of several days at 0-4.degree. C., when its concentration is 1-2 mg/ml and under moderate ionic strength conditions (Wingfield et al., 1991). Addition of protein B23 also completely prevented this time-dependent Rev polymerization. Thus, the ability of protein B23 to inhibit Rev aggregation can be seen under several different conditions of ionic strengths and temperature.

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L2: Entry 67 of 270 File: USPT Mar 6, 2001

DOCUMENT-IDENTIFIER: US 6197583 B1
TITLE: Therapeutic compounds

Drawing Description Text (5):
FIG. 3 compare helical wheel diagrams illustrating Nef and melittin, showing the distribution of polar and hydrophobic residues around the .alpha.-helices of the two peptides;

Drawing Description Text (10):
FIG. 8 depicts a molecular model showing monomeric Nef peptide with its hydrophobic region inserted in the hydrophobic region of a lipid binary, and its polar residues oriented to the external aqueous medium;

Detailed Description Text (4):
Sequences encoding Nef 27 and Nef 25 were amplified by PCR from the HIV-1 infectious clone pNL4.3 (Adachi et al, J. Virol. 1986 59 284-291) and subcloned directly into E. coli, yeast and baculovirus expression vectors. The scheme employed is summarised in FIG. 1. The yeast and baculovirus-derived Nef had native N-termini, but the expression levels were low. The expression level of the E. coli-derived glutathione (GST)-Nef fusion proteins was very high, and a major portion of the expression products was soluble. Large-scale production of E. coli-derived Nef 27 and Nef 25 was carried out by growing recombinant cells in a fermenter under fed-batch conditions. The soluble GST-Nef fusion proteins were affinity purified on glutathione-Sepharose. After thrombin cleavage at the fusion junction, the GST was selectively removed by binding it to glutathione-Sepharose. Under conditions of large-scale production Nef 27 was always contaminated with a slightly smaller N-terminal cleavage product, and the two could be resolved by selective binding of intact Nef 27 to a Reactive-Red 120 dye ligand. A final gel filtration step was sometimes necessary to remove traces of contaminating bacterial proteins. After purification Nef 27 and Nef 25 had the expected N-terminal sequences, and appeared as single monomeric bands on reducing SDS-PAGE and on gel filtration. Under non-reducing conditions both Nef 27 and Nef 25 existed as a mixture of monomers and dimers. The highly purified Nef proteins had no G-protein activities; neither Nef 27 nor Nef 25 had detectable GTP or autophosphorylation activity. Although both proteins showed appreciable GTP binding when compared to bovine serum albumin, chymotrypsin or lysozyme, the level of binding was insignificant compared to that shown by p21.sup.ras.

Detailed Description Text (20):
Theoretical calculations were carried out to assess the ability of the Nef peptide to assume a defined secondary structure, such as .alpha.-helix or .beta.-strand. Chou-Fasman analysis suggested a low probability of .alpha.-helix formation in aqueous solution, and hydrophobic moment plots suggested that the peptide was close to the boundary between surface seeking and "globular" behavious. Plots of hydrophobic moment against hydrophobicity are given in FIG. 2. FIG. 2A was plotted using a 6 residue `window` and FIG. 2B was plotted using a 4 residue `window`. It was considered possible that, like melitting, the Nef peptide might form an .alpha.-helix in the appropriate environment, such as the interface between a lipid bilayer and aqueous medium.

Schiffer-Edmundson diagrams for both peptides are given in FIG. 3. It can be seen that the Nef peptide has a considerably smaller arc than does melittin of hydrophobic residues which could interact with the hydrocarbon region of a lipid bilayer.

Detailed Description Text (24):
An energy-minimised structure of a representative Nef peptide representing several different strains of Nef is compared with the structure of melittin in FIG. 4. A set of coordinates for the structure of the latter is available from NMR studies. The Nef structure was assembled applying .alpha.-helical constraints, and energy minimised using the Discover program. Both the structures are presented with the hydrophobic residues facing downwards. Like melittin, the Nef peptide has a proline residue, approximately half-way along the sequence, which produces a characteristic kink in the helix. A striking difference between melittin and Nef is the fact that while the hydrophobic residues of the former lie along the concave face of the helix, those of the latter lie along its convex face.